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Prevalence, serotypes, and third generation antibiotic-resistant patterns of Salmonella enteric serotype Typhi, in Peshawar

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***Abstract*---**Most of the major health problems are caused by *Selmonella* virus. This virus is recognized as the most common food born pathogen. We documented the participants' health conditions, including their demography, antibacterial sensitivity, and epigenetic changes for resistance to distinct *Salmonella. Enterica* variants, isolated here in 33 different strains of *Salmonella Enterica* were identified through biochemical characteristics and 16S recombinant deoxyribonucleic acid sequence. The most frequent bacterium, *Salmonella Enterica Serovar Enteritidis*, caused 39.4% of incidents, afterward, 21.2% were caused by *Salmonella Paratyphi species*, 15.2 percent by *Salmonella Typhimurium* species, 12.1% by *Salmonella Typhi* species, and 12.1% by *Salmonella Arizona* species. Most of the isolates demonstrated resistance to 1st-2nd generation cephalospori. A

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few *Salmonella Enterica* strains were also susceptible to the primary antimicrobial drugs salmonellosis, such as, chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole. Additionally, the results showed the emergence of two *Salmonella Enterica* strains that were sensitive to 3rd generation cephalosporins. In *Salmonella Enterica* strains, the existence of carb-like, dihydrofolate reductase1 (dfrA1), florfenicol resistance genes (floR), and Tetracycline resistance protein A (tetA) genes, respectively, was accompanied with acclimation to different antibacterial drugs (chloramphenicol, tetracycline, B-lactam drugs and trimethoprim-sulfamethoxazole). These findings advance our knowledge of *Salmonella* that is food-borne announcing the emergence of *Salmonella Enterica* extracts that are sensitive to multiple drugs and offering essential information on multi-drug sensitivity.

Keywords---*Salmonella*, food-borne disease, antibiotics, multi-drug sensitivity

Introduction

Salmonellosis infection caused by *Salmonella* are considered to be some of the worldwide health foodborne disease, impacting a large diversity of victims despite major advancements in drinking and eating quality.[1] There are two main kinds of *Salmonella* species, *Salmonella Bongori* and *Selmonella Enterica*, which grow in presence or absence of oxygen (Facultative anaerobe) internalised gram-neg flagellated bacilli. *Salmonella Enterica* may be divided into more than twenty-five hundred groups depending on antigenic alterations in the antigen (Lipopolysaccharide-O).[2] The maximum among those are thought to be significant human pathogens. *Salmonella* can induce two primary types of illnesses. Enteric fever, gastroenteritis, and bacteremia are the symptoms of invasive typhoidal salmonellosis (ITS), which is caused by *Salmonella Enterica* serotype *Typhi* and *Paratyphi* A, B, and C; (ii) non-typhoidal salmonellosis (NTS) and (iii) enteric fever. Humans frequently get infections by *Salmonella* because of the consumption of infected drinking water and food, primarily dairy products; but, typhoidal *Salmonella*, which affects only humans, is spread via the fecal - oral transmission route or via intimate interaction with ill people.[3,4]

Salmonella Enterica variants resistance to multiple drugs (quinolones, fluoroquinolones and cephalosporin) which are the preferred medications for treating severe salmonellosis infection, have emerged as a consequence of the misinterpretation of antibacterial drugs in people and domestic animals, continue to pose a serious global health concern.[5-7] Horizontal resistance genes have spread throughout bacterial species as a result of the extensive use of antibacterial medications for treating diseases (human and animals).[8] The propagation of sensitive genes among microbial populations. Stimulation of antibiotic efflux systems, release of hazardous of specific antimicrobial substances, cell responsiveness to drugs, changes in the site of active substance, and other factors all contribute to bacterial resistance in *Salmonella Enterica*.[9] This research sought to determine the most prevalent *Salmonella* haplotype,

investigate how drug resistance spread across *Salmonella* strains, and investigate the genetics of resistant pathogens in isolated strains.

Methodology

Healthcare Data Collection

Clinical samples of various types were taken from people exhibiting signs of *Salmonella* infection. Clinical samples comprised faeces, urine, and blood. The Waste Disposal Facility provided many samples in Mardan Medical Complex (Pakistan). The samples were gathered sterilely and transported to the lab in a refrigerated box within 2-3 hours for separation of bacteria.

Detection and separation of microorganisms:

The clinical and environmental specimens were serially diluted (10-fold) in 1% sterile peptone water. To encourage the growth of *Salmonella* spp., every concentration was injected with 0.2 milliliter into the *Salmonella* culture. On new plates, *Salmonella* cultures were repeatedly collected and re-suspended until homogenous populations were formed. *Salmonella* cells were identified by their production of red-purple communities with dark black cores on Xylose Lysine Deoxycholate culture media and lack Lacto-fermentation light groups with black cores on deoxycholate citrate agar media. Gram staining techniques were used to confirm that the clusters were bacteria (Gram negative), and glycerol colonies of each strain were created, stored at 100 C, and ready for further research. Biochemical assays were used to identify the isolated bacterial strains. By genotyping the 16-S recombinant DNA sequence, the bacterium's identification was confirmed.

16-S analysis of recombinant DNA:

n = 33 *Salmonella* strains were added to nutritional medium. DNeasy Blood & Tissue Kits were used to extract total bacterial DNA. Purified genomic DNA from *Salmonella spp*. A template from isolates was employed for PCR technique. Following a 10-minute initial stage at 90^{o} C, the PCR method was done out in 45 phases that comprised denaturation at 90 degree Celsius aimed at 40 seconds, annealing on 54 degree celsius for 45 seconds, extension at 72 degree celsius aimed at 2.5 minutes, and lastly an extension at 72° c step (10min). Using a 1.5 kbp DNA ladder, a 1.0% agarose gel was used to separate the Polymerase Chain Reaction findings.

***Salmonella* serotyping:**

Slide agglutination tests were used to serotype the recovered *Salmonella* strains using available commercially mono O groups and poly O groups *Salmonella* A-E antisera. The collected *Salmonella*'s *Serovars* were identified using powerful and versatile *Salmonella* antisera against phase 1 and 2 flagellated H antigens, certain isolates (*Salmonella Typhi* and *Salmonella Paratyphi*) may show Vi, a capsular polysaccharide epitope that prevents the isolates from adhering to antisera-O. Consequently, the O-antigen was also found.

Antimicrobial susceptibility testing:

By using disc diffusion method, the *Salmonella* isolates were evaluated for susceptibility to 30 routinely used antimicrobial drugs. Afterward the overnight cultivation, the cells were extracted by means of only a disinfected swab and placed in a clean salt-water solution for 0.6 seconds. Cell suspensions were injected applying sanitized swabs, and numerous drugs discs were deposited on the Mueller-Hinton agar plates, before the plates were refrigerated for 24-48 hours at 37^{o} C.

Detection of antimicrobial resistance determinants:

With minimal modifications to an earlier method, PCR was used to identify numerous antimicrobial resistance genes in recovered *Salmonella Enterica*isolates. The carb-like, tem, and oxa-1 genes, which encode for sensitivity to β – antibiotics, were examined in the strains; the tetA, tetG, and tetB genes, which express tolerance to tetracycline; the floR gene, which encodes for tolerance to chloramphenicol; dfrA1 and A14 genes, the dfrB genes which encode for trimethoprim resistance. In a PCR, DNA was amplified under the following requirements: initial denaturation for 3 minutes at 95 degrees Celsius, then 40 cycles of denaturation for 2 minutes at 95 degrees Celsius, 35 seconds at the annealing temperature of every primer, extension for 2.5 minutes at 72 degrees Celsius, and final extension for 10 minutes at 72 degrees Celsius. The improved genes were evaluated using electrophoresis on 1-2% agarose gels. Additionally, the gyr-A and par-C genes.

Results

***Salmonella* detection and isolation**

A total of 100 non-repeating microbial isolates of *Salmonella* spp. were identified after the extraction and isolation of collected samples. A total of 33 isolates were classified as *Salmonella Enterica* depending on metabolic responses and biochemical characteristics. They were also oxidase negative and generated H2S. Additionally, all samples got tested for tryptophan, indole, amygdalin, Voges Proskauer and urea. The identification of the samples were verified using 16S rDNA gene sequencing in conjunction to biochemical testing. Many strains' 16S rDNA genes were successfully transcribed, cleaned, and decoded; their expected length was around 1525 bp. All isolates were categorised into several types of *Salmonella Enterica* subsp with 97–99% identity, and the genomes were submitted in GenBank with accession numbers. The phylogenetic tree shows how closely linked isolated *Salmonella Enterica* strains are to one another genetically depending on 16S rDNA data.

Table 1 – *Salmonella*'s many antibiotic resistant factors have unique primer sequences.

Drugs	Genes	Genome Sequence	Temperature for Annealing	Sequence size
Tetracycline	Tet (A,B,G)	F-GATATTCTGAGCACTGTCGC R-CTGCCTGGACAACATTGCTT F-TTGGTTAGGGGCATGGGTTG R-GTAATGGGTTGCTAACACCG F-GCTCGGTGGTATCTCTGC R-AGCAACAGAATCGGGAAC	58 60 55	1000 500 600
Beta-Lactams	Carb Tem oxa	F-AATGGTAGTCAGCGCTTCCC R-GGGGCTTGTAGCTCACTCCA F-TTGGTTGGACGAGTGGGTTA R-TCTTGGCTTTTATGCTTG	60 60 60 60	590 500 600
Chloramphenicol	floR	F-CACGTTCAGGGTCTATAT R-ATGCAGAAGTACTACGCG	50	870
Quinolone	Gyr-A Par-C	F-AAATGTACCCGTGTCGTTGGT R-GCCATAGGTACGGCGATAC	50 55	345 270 950

		C F- CTATGCGAGGTCAGAGCTG G R- TAACAGCACGACGGCGTAT T		
Trimethoprim	Dfr (A1, B and A14)	F- GTGAATGTATCACTAATGG R- TTAACGCTTATGCCAGATTT F- GATCACGTGCGCAAGAAA R- GCGCAGCCAGACGATAAAT F- GAGCAGGTICTIATIAAAGC R- TTAGCCCTTTIICCAAGGTT	50 60 60	450 150 340

Table 2 – Identification of the identified *Salmonella Enterica* isolates by serotype and serogroup

Sero-type		**kinds**	**No of extracts**	**Sero-group**
Salmonella Enterica	*S. Enteritidis*	NTS	10	D
	S. Arizonae		5	n/a
	S. Typhimurium		5	n/a
	S. Typhi	Typhoidal	5	n/a
	S. Paratyphi		5	B
	S. Paratyphi		5	C

Note: S for Serovar

The molecular basis of antibiotic resistance

The completely separate *Salmonella Enterica* isolates were tested by PCR for the existence of several antibiotic-resistant genes, including stetA, tet B and G for tetracycline and tem and oxa1 like genes for β-lactam antimicrobial drugs, the floR gene for chloramphenicol, the sdfrA1, dfr B, and A14 genes for trimethoprim, and the recognition of smutations in the results showed that the strains had a variety of susceptibility elements.

Sensitivity to ampicillin and piperacillin was only tested in four segregates, and three segregates displayed a severe arrangement of amoxicillin acid and clavulanic acid tolerance, respectively. Morphologically, 87-90 percent of the total of strains that are resilient to 1st and 2nd generation cephalosporins drugs. Only isolated strains were chloramphenicol tolerant despite the overwhelming number of isolates carrying the floR gene, which confers chloramphenicol resistance. All trimethoprim-sulfamethoxazole resistant isolates included the dfrA1 gene, but none contained the dfrB or A14 genes, showing that the dfrA1 gene mediates resistance to trimethoprim-sulfamethoxazole. Three isolates of *Salmonella Enterica* including *Serovar Paratyphi C*, and one isolate each of *Serovar Typhi* and *Arizonae*. These five isolates' amplified gyr-A and Par-C genes were disinfected, sequenced, and the outcomes were matched. The results showed that there were point alterations in the par-C gene at locations 13, 19, and 20, while there were point alterations in the gyr-A genes at positions 13 and 24.

Discussion

Different strains of Salmonella were detected which were found to be associated with Salmonellosis and considered to be a serious threat to public health, and both clinical as well as microbial incidence have been on the increase.[10-12] They were recovered and classified as *Salmonella Enterica* after 16S rDNA genotyping and molecular characterizations. Typhimurium made up 39 and 15 percent of the overall, typhoidal *Salmonella*, which included *S. Paratyphi* and *Typhi*, respectively, made up 12 and 21 percent, correspondingly.[13,14] Furthermore, *S. Arizonae* was found in 12% of the isolates. These results agreed with the findings of other international studies. Each and every *S. paratyphi B* strain tested positive for resistance to the primary antibiotics ampicillin and chloramphenicol. This resistance pattern lined up with other studies that were previously provided. Salmonellosis is considered to be a serious cause of morbidity and mortality, and both clinical and natural bacterial incidence have been on the rise.

Our findings showed the maximum exposure of *Salmonella* Typhi strains (81%) to both chloramphenicol and ampicillin, in contrast to other studies that showed substantial resistance of *Salmonella* Typhi strains to all primary therapies.[15-18] Additionally, erythromycin and nitrofurans were both resistance to all strains and the bulk of strains, correspondingly. *Salmonella* resistance to these medicines would be most likely caused by the use of nitrofurans and erythromycin by veterinarians, particularly in the livestock sector, as feed additives and/or treatments.[19] The results showed that two strains acquired resistance to 3rd generation cephalosporin drugs. Nevertheless, there's really evidence that some *Salmonella Enterica* isolates are becoming more resistant to quinolone drugs. In isolated with sensitivity to or reduced exposure to Beta-lactam antibiotics, evaluation of resistant strains in collected *Enterica* strains discovered the occurrence of a carb gene, suggesting that this resistance is transmitted by the carb-like gene, which encodes the B-lactamase enzyme. In contrast to previous investigations, no isolates had either the tem or the oxa-1 genes.[20-22]

Table 3 – Mechanisms of multi - drug resistance among *Salmonella Enterica* strains

***Salmonella* Enterica strains**	**Sero-type**	**Sensitivity**	**Remarks**
SA-10	SPC	FOX, TM,CF,FT,AN,CXM,SXT, GM and CXA	PQR
SA-7	SPC	SXT, FT, TM, FOX, AN, GM, CXA, CXM, CF	PQR
SA-14	ST	CXM, FOX, CXA, CF GM, CXA, CXM, CF	PQR
SAM	SPB	PIP, AM, CF, CXA, CXM, AN, FOX, GM, FT, TM	BLR
SA-12	SPC	CXM, CF, FOX, CXA, GM, AN, FT, SXT, TM	SR
ParaC	SPC	AM, FOX, PRP, CF, CXM, FOX, AN, SXT, TM, GM	BLR and PQR
NS-10	SA	PIP, AM, CXM, CF, CXA, CPD, GM, SXT	BLR and PQR
CR	SPB	CXM, CF, FOX, CXA, GM, AN, FT, SXT, TM	BLR

Note: Salmonella Paratyphi C (SPC), *Salmonella Typhi* (ST), *Salmonella Paratyphi B* (SPB) and *Salmonella Arizonae* (SA), Partially quinolones resistance (PQR), B-Lactams resistance (BLR), sulfa resistance (SR)

Conclusion:

We report epidemiological, drug sensitivity, and the genomic underpinnings of tolerance among the *Salmonella Enterica* isolates acquired in this study. exhibiting a 1st - 3rd generation cephalosporin sensitivity. It also outlines some characteristics of the recently found molecular mechanism of resistance and confirms the notion that *Salmonella Enterica's* antibiotic resistant mechanism changes depending on the specific geographical area and isolation surroundings. To learn more about Salmonellosis, additional in-depth analysis of large sample sizes will be done using the data gathered.

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